with rotamase activity and the conversion of the substrate is detected after a period of time. The method for detecting conversion of the substrate will vary with the particular substrate chosen. One method has been termed the Ki test (See Harding, et al., Nature, 341:758-760 (1989). The cis-trans isomerization of an alanine-proline bond in a model substrate, N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide (SEQ ID NO: 1), is monitored spectrophotometrically in a chymotrypsin-coupled assay. The action of chymotrypsin releases p-nitroaniline from only the trans form of the substrate. The amount of p-nitroaniline can be monitored in a spectrophotometer, for example. Other methods of detecting the presence of p-nitroaniline can also be used. The inhibition of this reaction caused by different concentrations of inhibitor is determined and the data are analyzed as a change in first-order rate constant as a function of inhibitor concentration, which yields the Ki value.

The following were added to a plastic cuvette: 950 mL of ice cold assay buffer (25 mM HEPES, pH 7.8, 100 mM NaCl), 10 μL of CyP A (2.5 μM in 10 mM Tris-Cl pH 7.5, 100 mM NaCl, 1 mM dithiothreitol), 25 μL of chymotrypsin (50 mg/ml in 1 mM HCl), and 10 μL of test compound, at various concentrations, in dimethyl sulfoxide. The reaction was initiated by the addition of 5 μL of substrate (succinyl-Ala-Phe-Pro-Phe-para-nitroanilide (SEQ ID NO: 1), 5 mg/mL in 470 mM LiCl in trifluoroethanol). The absorbance at 390 nm versus time was monitored for 90 seconds using a spectrophotometer and the rate constants were determined from the absorbance versus time data files.

The IC50 values that were obtained for representative compounds in the following Table I refer to the concentration that inhibits 50% of the rotamase activity in a sample. The lower the value, the more active the compound is at binding or interacting with CyP. The Cyclophilin utilized was a recombinant rat CyPA-GST fusion protein: CypA was amplified from rat brain cDNA using standard PCR methods, primed with the following sequences: 5' CCC CCC GGG AGT CAA CCC CAC CGT GTT CTT CGA 3' (SEQ ID NO: 2) and 5' GGA GAT CTA GAG TTG TCC ACA GTC GGA GAT GGT 3' (SEQ ID NO: 3). The resulting fragment (573 base pairs) was cloned into pCRII and amplified. The CyP sequence was cut out with SmaI and EcoR1 and cloned into the SmaI/EcoR1 sites in pGEX2TK (Pharmacia). This plasmid was transformed into BL21 E. coli cells for expression of the GST-CyPA fusion protein. An asterisk indicates that the compound was evaluated using a human recombinant CyPA, [Yoo et al., J. Mol. Biol., 269 (1997) 780-95].

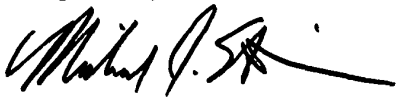
Remarks

Applicants amend the specification to add a Sequence Listing and to refer to SEQ ID NOs, as required by 37 C.F.R. §§ 1.821-825. A computer-readable form of the Sequence Listing is also enclosed. No new matter enters by the amendment.

In accordance with 37 C.F.R. § 1.821(f), the undersigned hereby states that the paper copy of the enclosed Sequence Listing and the computer-readable copy of the enclosed Sequence Listing are the same. Further, as required under 37 C.F.R. § 1.821(g), the undersigned hereby states that this submission includes no new matter.

Applicants believe that they have adequately provided for any extensions of time or required fees or petitions in order to have this paper considered and/or keep the application pending. However, if extensions of time or any other fees or petitions are necessary, then applicants hereby petition, under 37 C.F.R. § 1.136(a) or any other rule, and the fees therefor (including fees for net addition of claims or other petition fees) are hereby authorized to be charged to our Deposit Account No. 08-3038, referencing docket number 03166.0029.NPUS02.

Respectfully submitted,



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